Figure 1 - Reina-San-Martin, B. *et al.*

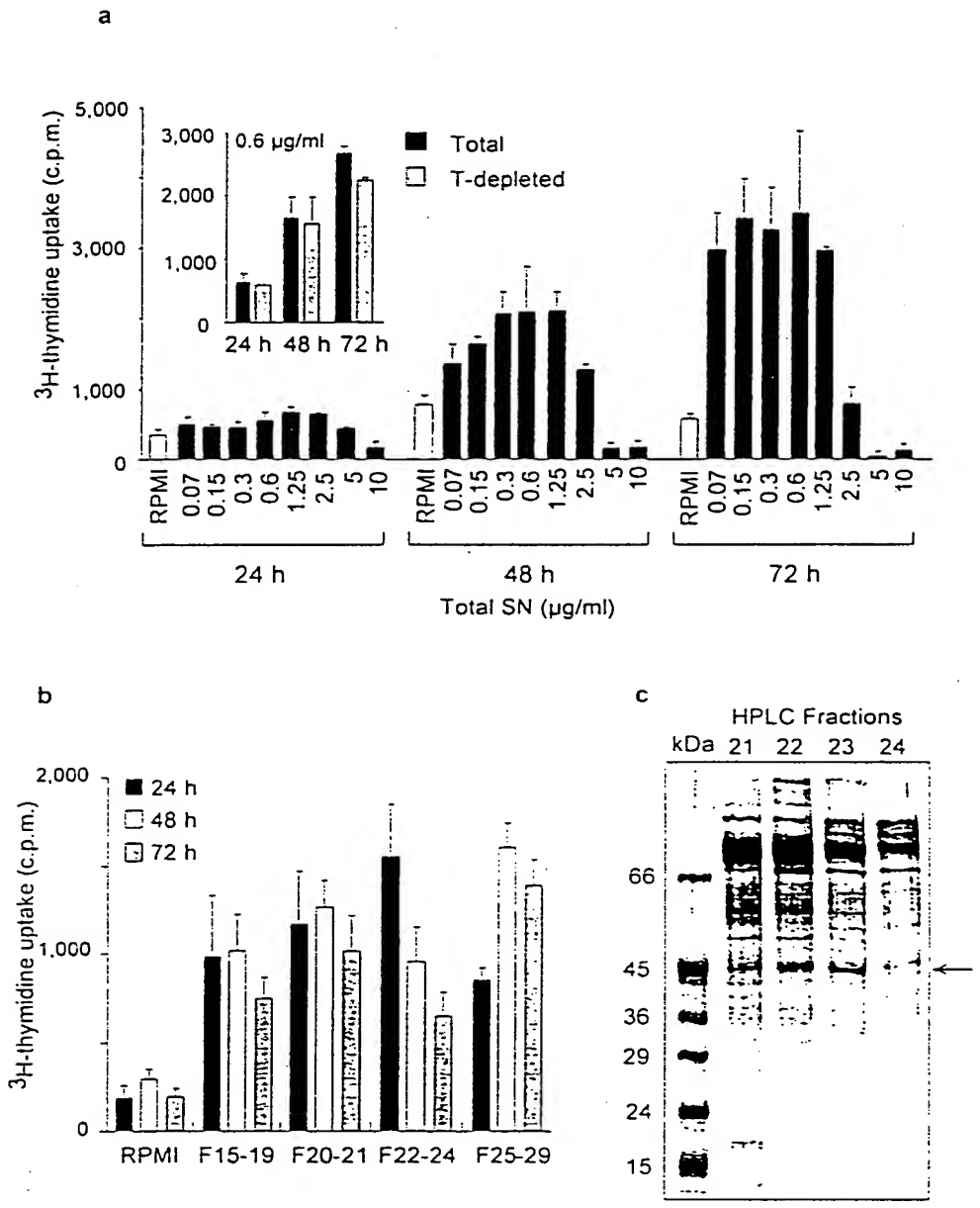


Figure 2 - Reina-San-Martin, B. *et al.*


```
Tc  MRKSVCPKQKFFFSAFPFFFFFCVFPLISRTGQEKLLFDQKYKIIKGEKKEKKKNQRANRREHQQKREIMRFKKS  75
Cs  ------------------------------------------------------------------MKFSKG   6
Pa  ------------------------------------------------------------------MQR    3

Tc  FTCIDMHTEGEAARIVTSGLPHIPGSNMAEKKAYLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEEGADLGMVF 150
Cs  IHAIDSHTMGEPTRIVVGGIPQINGETMADKKKYLEDNLDYVRTALMHEPRGHNDMFGSIITSSNNKEADFGIIF  81
Pa  IRIIDSHTGGEPTRLVIGGFPDLGQGDMAERRRLLGERHDAWRAACILEPRGSDVLVGALLCAPVDPEACAGVIF  78

Tc  MDTGGYLNMCGHNSIAAVTAAVETGIVSVPAKATNVPVVLDTPAGLVRGTAHLQSGTESEVSNASIINVPSFLYQ 225
Cs  MDGGGYLNMCGHGSIGAATVAVETGMVEMVEPVTNIN--MEAPAGLIKAKVMVEN---EKVKEVSITNVPSFLYM 151
Pa  FNNSGYLGMCGHGTIGLVASLAHLGRIGPGV------HRIETPVGEVEATLH-------EDGSVSVRNVPAYRYR 140

Tc  QDVVVVLPKPYGEVRVDIAFGGNFFAIVPAEQLGIDISVQNLSRLQEAGELLRTEINRSVKVQHPQLPHINTVDC 300
Cs  EDAKLEVPSLNKTITFDISFGGSFFAIIHAKELGVKVETSQVDVLKKLGIEIRDLINEKIKVQHPELEHIKTVDL 226
Pa  RQVSVEVPGI-GRVSGDIAWGGNWFFLVAGH--GQRLAGDNLDALTAYTVAVQQALDD----QDIRGEDGGAIDH 208

Tc  VEIYGPPTNPEANYKNVVIFGNRQADR SPCGT GTSAKMATLYAKGQLRIGETFVYESILGSLFQGRV--LGEE 371
Cs  VEIYDEPSNPEATYKNVVIFGQGQVDR SPCGT GTSAKLATLYKKGHLKIDEKFVYESITGTMFKGRV--LEET 297
Pa  IELFAD--DPHADSRNFVLCPGKAYDR SPCGT GTSAKLACLAADGKLLPGQPWRQASVIGSQFEGRYEWLDGQ 279

Tc  RIPGVKVPVTKDAEEGMLVVTAEITGKAFIMGFNTMLFDPTDPFKNGFTLKQ* 423
Cs  KVGEFD-----------AIIPEITGGAYITGFNHFVIDPEDPLKYGFTV*-- 335
Pa  PGGPIVPTIRGRAHVSAEATLLLADDDPFAWGIRR*---------------- 314
```

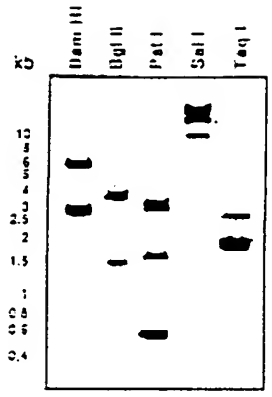


FIGURE 3(a)

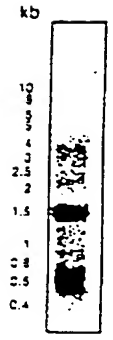


FIGURE 3(b)

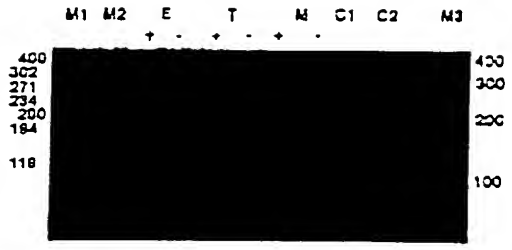


FIGURE 3(c)

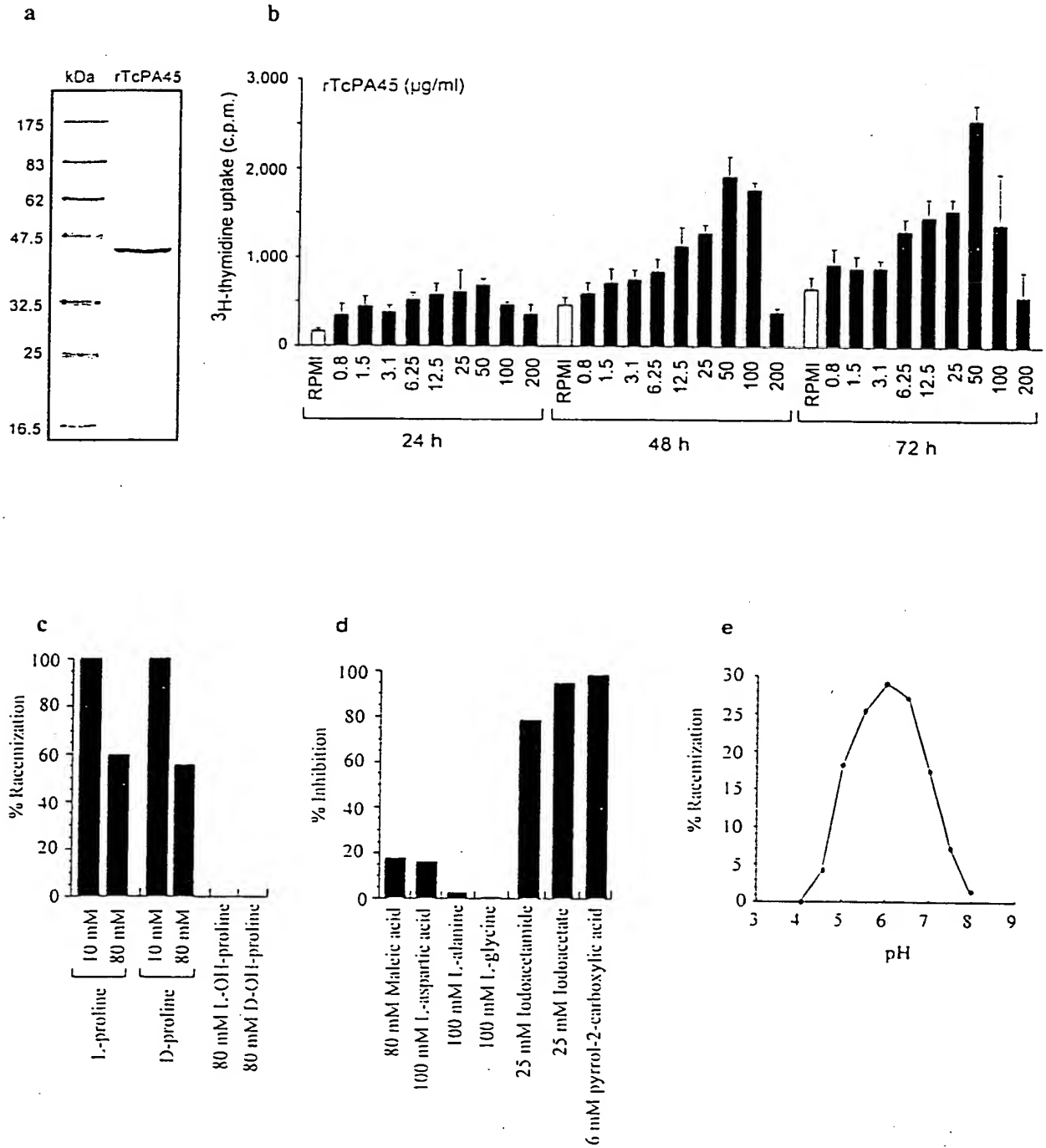
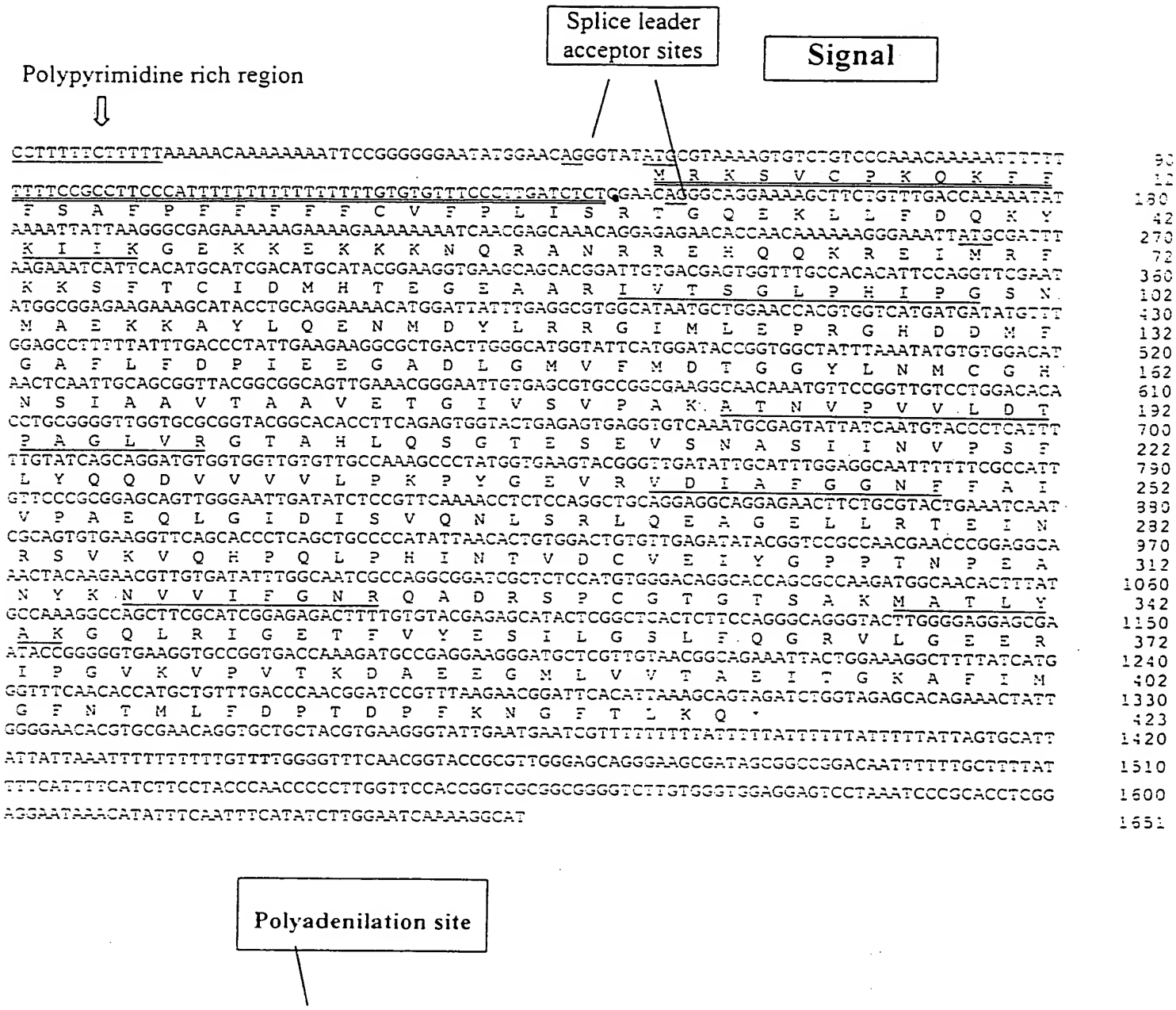

a
kDa
rTcPA45
175
83
62
47.5
32.5
25
16.5
b
rTcPA45 (µg/ml)
3H-thymidine uptake (c.p.m.)
3.000
2,000
1,000
0
RPMI
0.8
1.5
3.1
6.25
12.5
25
50
100
200
24 h
48 h
72 h
c
% Racemization
100
80
60
40
20
0
10 mM
80 mM
L-proline
D-proline
80 mM L-OH-proline
80 mM D-OH-proline
d
% Inhibition
80 mM Maleic acid
100 mM L-aspartic acid
100 mM L-alanine
100 mM L-glycine
25 mM Iodoacetamide
25 mM Iodoacetate
6 mM pyrrol-2-carboxylic acid
e
% Racemization
30
25
20
15
10
5
0
3
4
5
6
7
8
9
pH

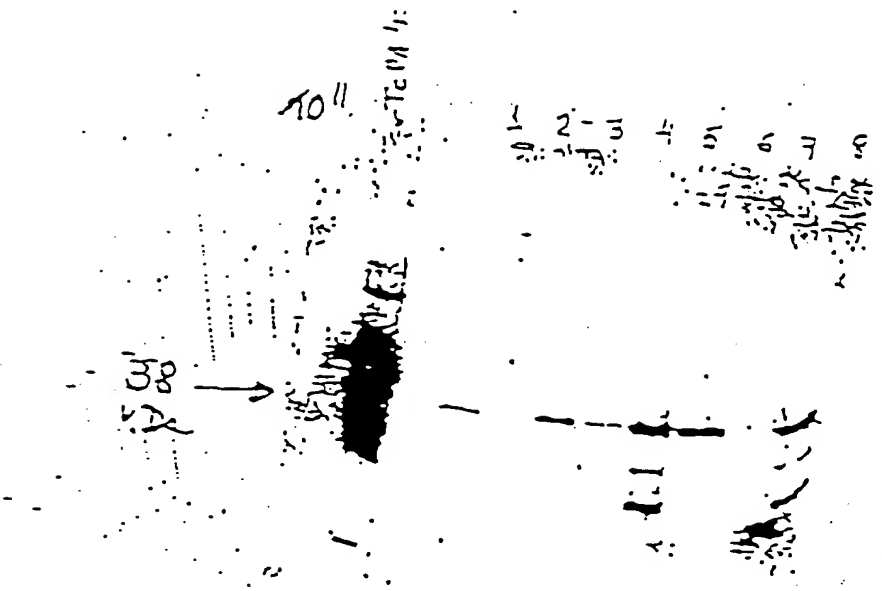
FIGURE 4



Obs : Underlined the sequenced peptides used to deduce degenerated primers for cloning

Nucleotide sequence and peptide sequence TcPA45

FIGURE 5



Western blot

A

LANE 3

SOLUBLE FRACTION OF EPIMASTIGOTE EXTRACT (CYTOSOLIC) REVEALED WITH ANTIBODY DIRECTED TO rTcPA45

⇒ DEMONSTRATE THE EXISTANCE OF A INTRACYTOPLASMIC FORM OF TcPA45 IN THE PARASITE

FIGURE 6

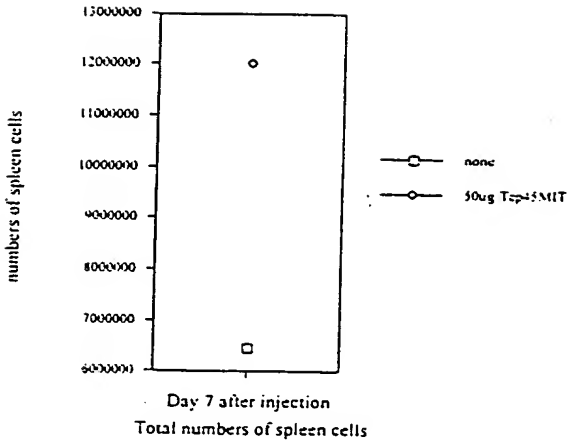

numbers of spleen cells
13000000
12000000
11000000
10000000
9000000
8000000
7000000
6000000
none
50ug Tag45MIT
Day 7 after injection
Total numbers of spleen cells

FIGURE 7

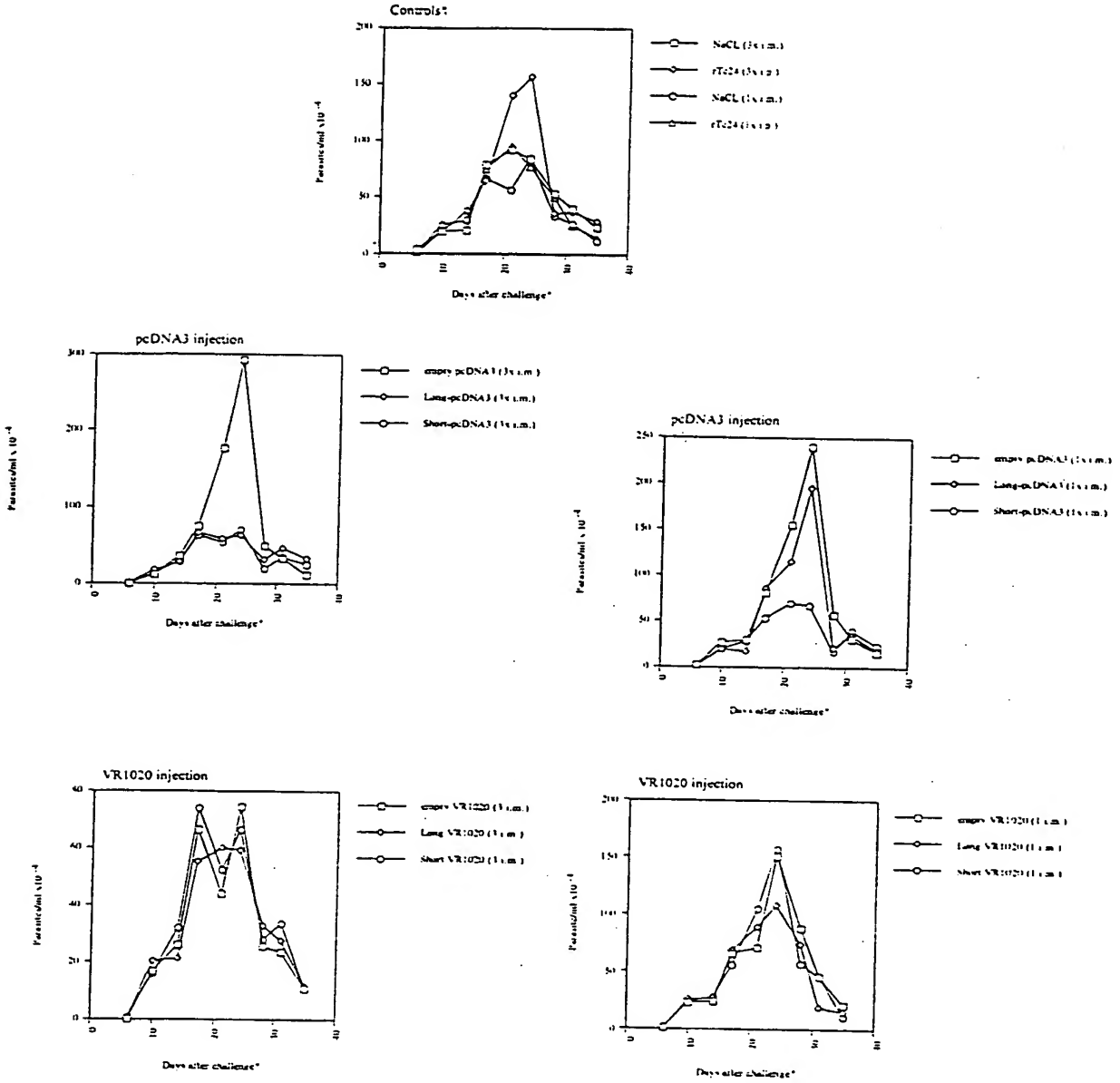

Controls
pcDNA3 injection
pcDNA3 injection
VR1020 injection
VR1020 injection

FIGURE 8

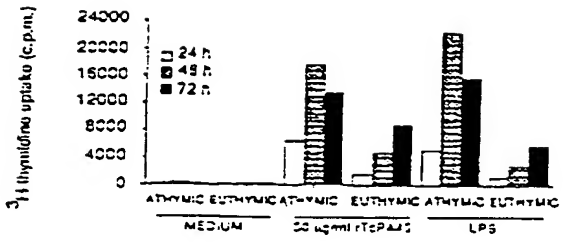

24000
20000
16000
12000
8000
4000
0
24 h
48 h
72 h
ATHYMIC EUTHYMIC ATHYMIC EUTHYMIC ATHYMIC EUTHYMIC
MEDIUM
LPS

FIGURE 9

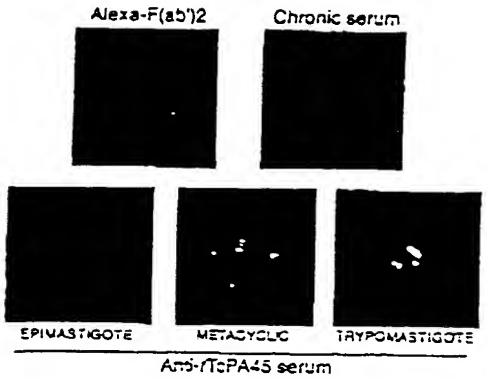

Alexa-F(ab')2
Chronic serum
EPIMASTIGOTE
METACYCLIC
TRYPOMASTIGOTE
Anti-rTcPA45 serum

FIGURE 10(a)

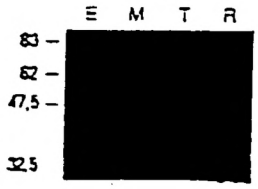


FIGURE 10(b)

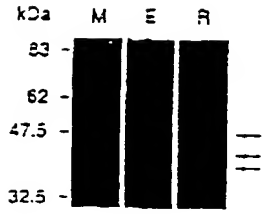


FIGURE 10(c)

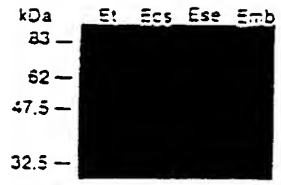


FIGURE 10(d)

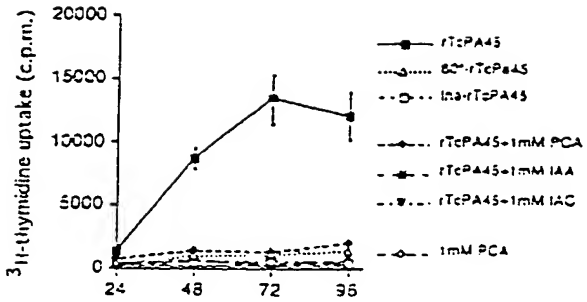

20000
15000
10000
5000
0
24
48
72
96
rTcPA45
rTcPA45+1mM PCA
rTcPA45+1mM IAA
rTcPA45+1mM IAC
1mM PCA

FIGURE 11(a)

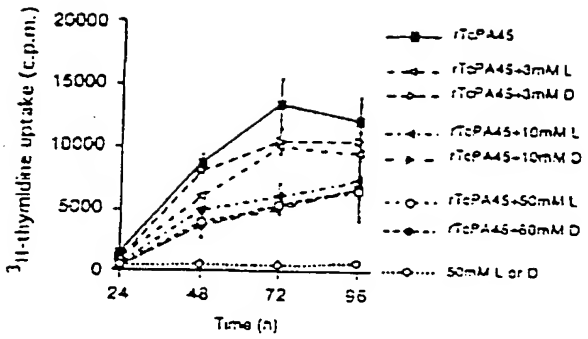

20000
15000
10000
5000
0
24
48
72
96
Time (h)
rTcPA45
rTcPA45+3mM L
rTcPA45+3mM D
rTcPA45+10mM L
rTcPA45+10mM D
rTcPA45+50mM L
rTcPA45+60mM D
50mM L or D

FIGURE 11(b)

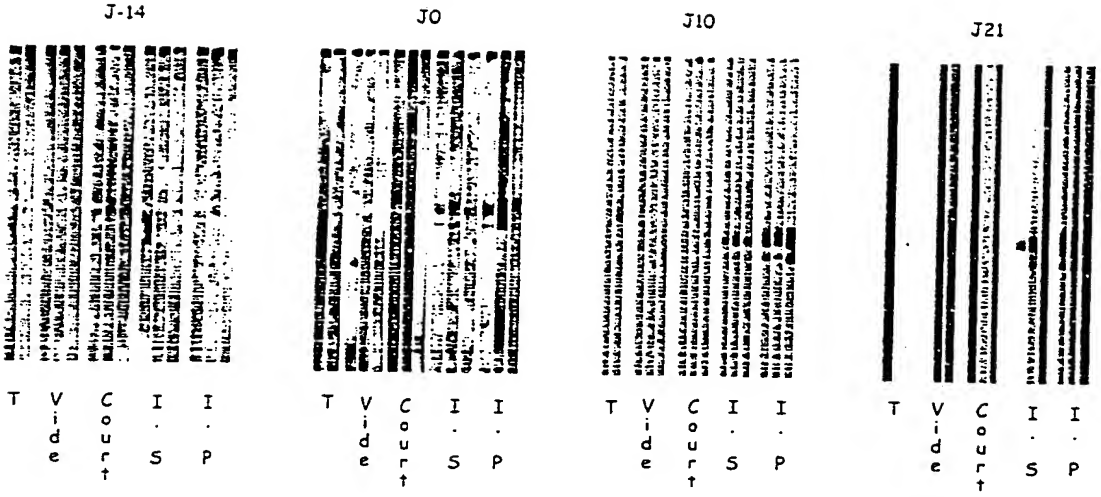

J-14
T
Vide
Court
I.S
I.P
JO
T
Vide
Court
I.S
I.P
J10
T
Vide
Court
I.S
I.P
J21
T
Vide
Court
I.S
I.P

FIGURE 12

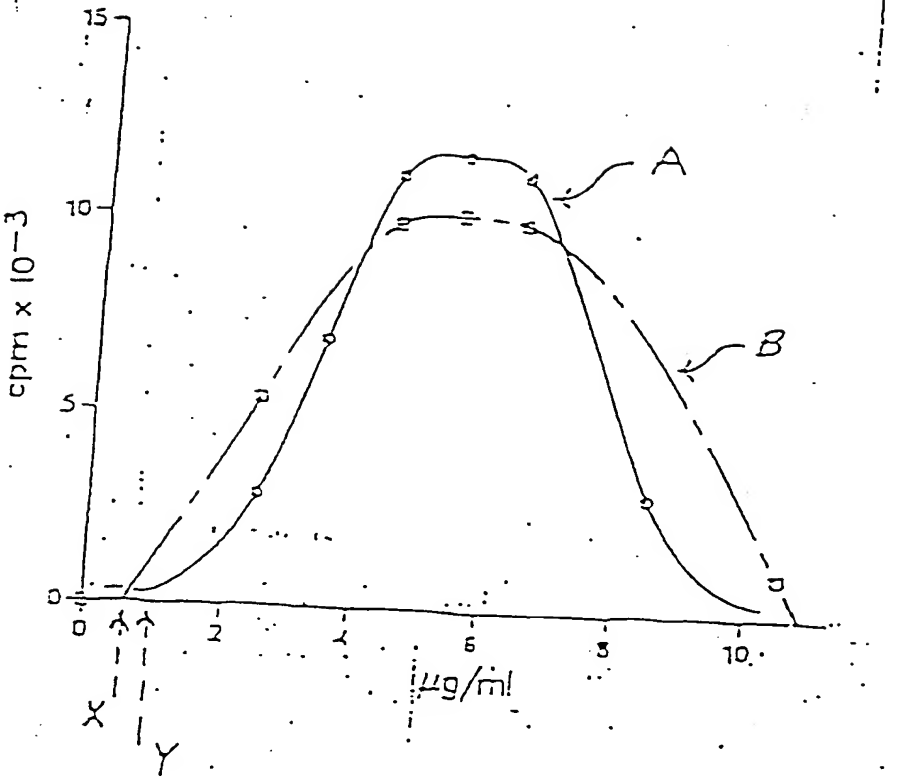


FIGURE 13

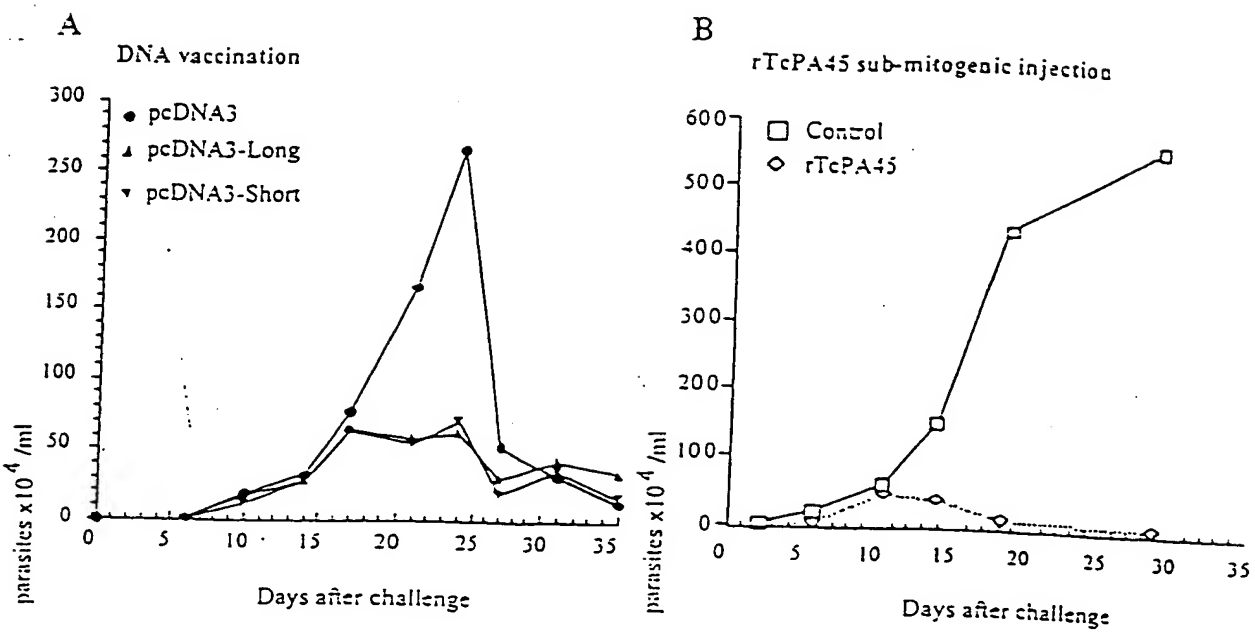

A
DNA vaccination
pcDNA3
pcDNA3-Long
pcDNA3-Short
parasites x10^4 /ml
Days after challenge
B
rTcPA45 sub-mitogenic injection
Control
rTcPA45
parasites x10^4 /ml
Days after challenge

FIG. 14